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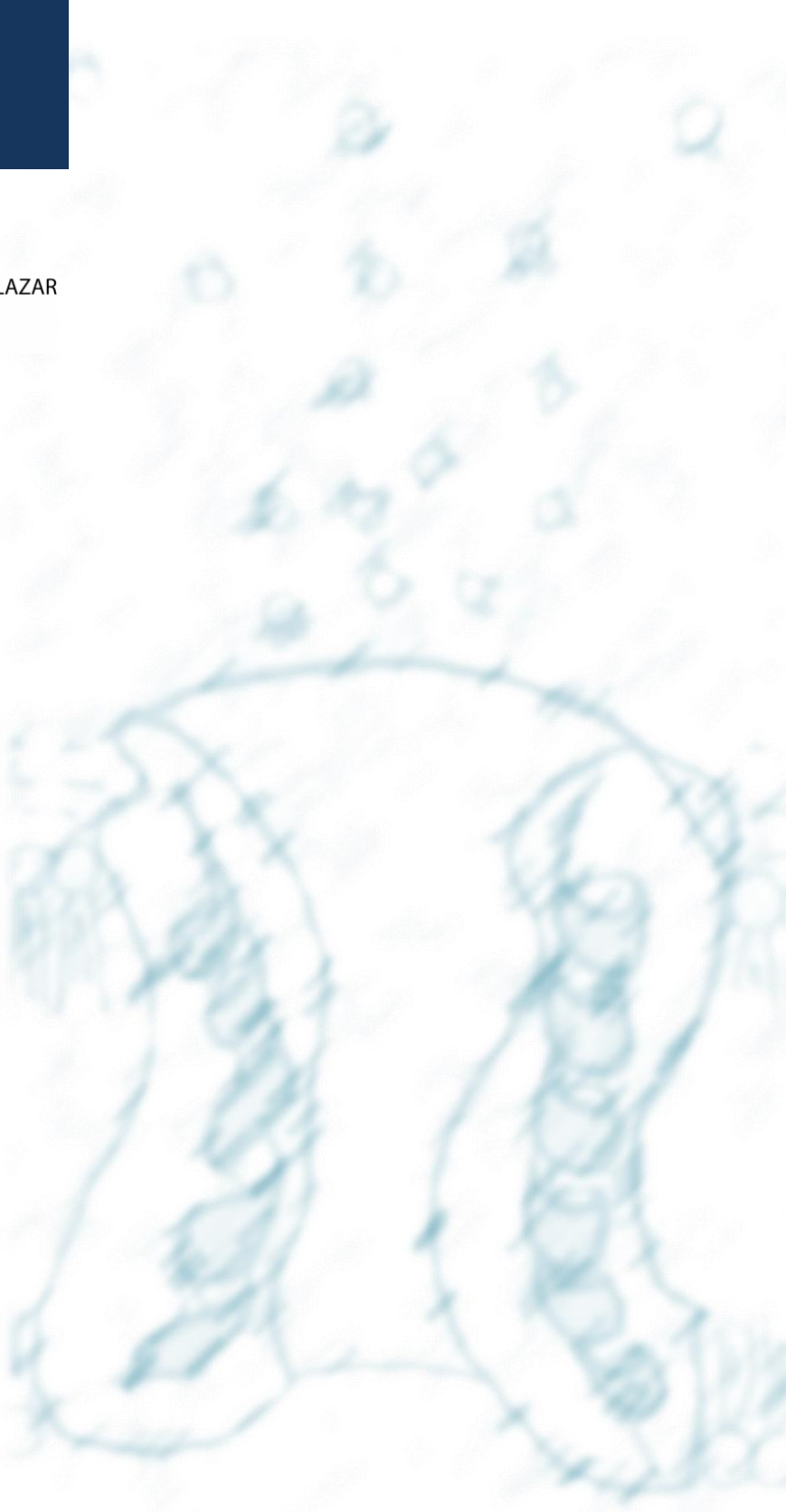

[AQUAPORINS: PATHOPHYSIOLOGY AND THERAPEUTICAL IMPACT IN PERITONEAL DIALYSIS]

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Review Article

AQUAPORINS: PATHOPHYSIOLOGY AND THERAPEUTICAL IMPACT IN PERITONEAL DIALYSIS:

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ABSTRACT

Introduction: In peritoneal dialysis (PD), convective and diffusive transport and osmosis are created through the peritoneal membrane in order to replace a faulty renal function. The transport across the membrane involves a rich microvascular network with paramount importance in the exchange process and rate. Aquaporins (AQP) are protein channels present in the capillaries walls, which facilitate the passive flux of water in presence of osmotic pressure, corresponding to the ultrasmall pore hypothesized by Rippe *et al.* responsible for the free water transport (FWT). The importance of aquaporins is undeniable, but the exact role of aquaporins in the pathophysiology of peritoneal dialysis and underlying processes such as ultrafiltration failure, inflammation, fibrosis and neoangiogenesis remains unclear.

Objectives: The aim of this work is to review the structure and functioning of aquaporins, their contribution in peritoneal physiology and potential as a pharmacological target.

Discussion: the discovery of aquaporins represented a breakthrough in human physiology, particularly in peritoneal dialysis. They represent not only a part of the explanation of water transport but an entirely new piece in the puzzling peritoneal transport and dysfunction, where their relation with other key processes and players such as a mesothelial cells, extracellular matrix and capillary network is not clear and deserves special attention. With a substantial share of knowledge of the channel provided by *in silico* models, KO null mice and *in vitro* cell culture, the clinical monitorization of aquaporin function will lead to a more integrated and accurate estimative of the aquaporin importance in prognosis and outcome of patients in peritoneal dialysis.
The modulation of aquaporins is possible but the compounds discovered so far, (transition metals) are too toxic for a safe use in clinical practice. The recent advance of *in vitro* monitoring of cell swelling provided the screening of compound libraries in a systematic way, and discovery of new molecules with promising results. The discovery of an aquaporin agonist and the possibility of modulation therapy could mean a significant shift in peritoneal dialysis, with the expectation of an even more efficient and prolonged technique.

Conclusions: Peritoneal dialysis represents an attractive form of renal replacement. More biocompatible peritoneal dialysis solutions and remodeling blockers are needed to a long-lasting technique. Aquaporins represent a major role in the pathophysiology of the peritoneal barrier, as the ultrasmall pore and in the pathological changes observed during peritoneal dialysis since it's involved in various processes like cell migration, angiogenesis and inflammation. Their study will bring further knowledge relevant not only to peritoneal dialysis, but to human physiology and cell biology.

Keywords: Aquaporins; AQP1; Peritoneal Dialysis; Ultrafiltration; Water transport

RESUMO EXTENDIDO

Desde a sua formulação enquanto hipótese teórica nos anos 80 até à demonstração da sua existência enquanto canal condutor de água, as aquaporinas têm sido alvo de investigação intensa que contribuiu para a evolução do papel primordialmente atribuído de canal passivo de fluxo de água.

As aquaporinas são uma família de proteínas transmembranares que se encontram divididas em 3 grupos consoante as suas capacidades de conduzir solutos, água e glicerol: aquaporinas ortodoxas, aquagliceroporinas e aquaporinas não ortodoxas. Nos mamíferos são reconhecidas 13 isoformas com diferenças a nível funcional e filogenéticas condicionadas por uma diferente codificação genética e ultra-estrutura.

Estruturalmente são tetrâmeros compostos por quatro monómeros. Cada monómero é formado por seis segmentos helicais e respectivas ansas de comprimento variável. A ligação entre hélices dá origem a uma estrutura semelhante a uma ampulheta com o motivo NPA no centro. Esta ligação é estabilizada pela tetramerização e empacotamento das hélices. A orientação das hélices e dos domínios extracelulares condiciona a conformação final da aquaporina e a sua selectividade à água. A alteração destes domínios leva a alterações drásticas da capacidade de condução podendo mesmo resultar num poro não funcionante. Baseado neste facto, uma das metodologias de estudo de aquaporinas consiste na alteração de resíduos por mutagénese e observação das alterações na capacidade de condução, permitindo estabelecer a importância de determinados resíduos e explicar as diferenças entre AQPs. A alteração estrutural por factores extrínsecos é denominada *gating* e tem como principio a existência de diferentes estados conformacionais.

As características do canal, nomeadamente a sua geometria e existência de dois locais de constrição (ar/R e NPA) tornam o canal selectivo para água: pelo diâmetro do poro e pela criação de uma barreira energética.

Relativamente às isoformas das aquaporinas e aquagliceroporinas, a maior diferença reside no diâmetro do poro, 2.8A e 3.4A, respectivamente.

A regulação destes canais ainda não se encontra completamente esclarecida, no entanto os principais mecanismos propostos são transcrição, transporte membranar e *gating.*

O último já comprovado em AQP vegetais mas ainda discutido em AQPs humanas.

Muito do conhecimento do transporte membranar deriva do modelo mais conhecido de controlo da AQP2 no rim. Neste a acção da vasopressina e subsequente activação da proteína cinase A, leva à migração e fusão de vesículas com AQP2 para a membrana. Recentemente, foi proposto um mecanismo de translocação para AQP1 em resposta a meios hipotónicos, que cursa com elevação do cálcio intracelular e fosforilação da AQP1 para a membrana em 30s.

A presença e distribuição destes canais varia conforme o órgão, porém o seu papel no endotélio e epitélio já era esperado, contudo o mesmo não se poderá dizer das funções recentemente atribuídas na migração e transmissão de impulso nervoso. No epitélio, contribui para o transporte de fluidos de diferentes modos: fluxo quase isosmolar e ampliação de fluxo no transporte activo. No rim é importante no transporte de água e na permeabilidade de determinados segmentos do nefrónio. A ausência de AQP1 em ratos AQP1 nulos, resulta numa disfunção urinária grave, com poliúria e incapacidade de concentrar urina.

No cérebro, a AQP4 funciona como um canal bidireccional e em ratos AQP4 nulos foram observadas taxas de sobrevivência díspares em resposta a diferentes tipos de edemas induzidos.

Outros estudos experimentais demonstraram uma participação inesperada das aquaporinas noutros fenómenos fisiológicos: migração celular, proliferação celular, metabolismo de lípidos e hidratação da pele.

As Aquaporinopatias são doenças caracterizadas por uma disfunção de aquaporinas , os mecanismos até agora conhecidos são: mutações com perda de função e resposta imune contra os epitopos extracelulares da APQ4. Mutações com perda de função são extremamente raras mas foram registados casos de diabetes insípida nefrogénica (AQP2) e cataratas congénitas (AQP0) atribuíveis à perda de aquaporinas. Por outro lado, a neuromielite óptica é um distúrbio auto-imune com afecção de aquaporinas por dano mediado por anticorpos específicos.

A associação entre polimorfismo de aquaporinas e patologias especificas ainda não se encontra descrito.

A diálise peritoneal apresenta-se como uma técnica de substituição renal com uma sobrevivência equivalente a hemodiálise, que pela sua relativa simplicidade, menores custos e melhor qualidade de vida dos pacientes é considerada como vantajosa.

A diálise peritoneal consiste assim na utilização da cavidade peritioneal e respectiva membrana peritoneal como interface de transporte entre solução de diálise e sangue, com extracção de água circulante e toxinas. Na práctica consiste na instalação de dialisado, um período de repouso e uma drenagem no fim do procedimento.

O transporte de solutos e água é obtido pela criação de gradientes osmóticos e de concentração entre sangue e dialisado, cuja composição inclui concentrações fisiológicas de iões (sódio, cloro, cálcio e magnésio), um agente osmótico (comummente glucose) e um composto tampão para estabilizar o pH da solução. Desta forma a água é extraída por osmose, pequenos solutos por difusão e macromoléculas por convecção.

O conceito de peritoneu, enquanto membrana e barreira de transporte é extremamente relevante para a diálise peritoneal e para os processos fisiopatológicos subjacentes.

O conceito anatómico de peritoneu difere do conceito de barreira de transporte em diálise peritoneal: nesta última para além do mesotélio e respectiva membrana basal, o interstício e microvasculatura são incluídos. Daí que a área total de peritoneu não corresponda a área que efectivamente participa nas trocas, uma vez que esta se encontra condicionada por outros factores.

A microvasculatura, formada por capilares contínuos e com capacidade auto-reguladora, é considerada a maior barreira de trocas, comportando-se com uma estrutura heteroporosa composta por poros de 3 tamanhos diferentes. Esta característica confere-lhe selectividade e influencia as taxas de transporte observadas. Os poros são divididos em grandes, pequenos e ultrapequenos.

A ultrafiltração, que corresponde a quantidade de água livre de solutos que atravessa a membrana, é um marcador preditivo de sobrevivência em doentes submetidos a diálise peritoneal. Segundo o modelo de Rippe, os ultrapequenos

poros são responsáveis pela ultrafiltração e correspondem morfologicamente as aquaporinas presentes no endotélio vascular.

Este achado explica o fenómeno observado na prática clinica de dissolução do sódio na primeira hora de diálise que foi atribuído à ultrafiltração pelas aquaporinas. A correspondência morfológica foi comprovada pela inibição de aquaporinas e uma perda de ultrafiltração sobreponível ao modelo teórico.

Na prática clinica, a quantificação de ultrafiltração atribuível às aquaporinas permite uma caracterização mais detalhada do perfil de transporte do doente.

A perda de função peritoneal é observada em doentes com PD de longa duração e deriva da utilização não fisiológica do peritoneu. O remodelamento peritoneal corresponde as alterações deletérias que são observadas em respostas a uma variedade de insultos, agudos e crónicos. Estas alterações traduzem-se em mudanças no microambiente celular com a sinalização celular predominantemente orientada para inflamação, fibrose e recrutamento de células.

Vários factores contribuem para este estado pró-inflamatório: a composição da solução da diálise, peritonite, o cateter e a uremia.

A glucose usada como agente osmótico nas soluções de diálise é considerada um dos factores determinantes no processo patológico, uma vez que os seus produtos de degradação vão induzir uma resposta inflamatória por parte das células mesoteliais com libertação de factores de crescimento, citocinas e recrutamento de células.

Apesar de não constituir um obstáculo ao transporte de água e solutos, o mesotélio desempenha um papel fundamental na resposta a estímulos como a diálise e peritonite. É capaz de sofrer transdiferenciação, e adquirir um perfil pró-fibrótico (miofibroblasto), bem como responder a estímulos de células vizinhas. A integridade do mesotélio reflecte o grau de dano a que o peritoneu foi submetido.

Todas estas alterações são sustentadas pela libertação de mediadores, factores de crescimento e citocinas. Destes destaca-se o TFG-β por induzir as alterações descritas no mesotélio e pela sua produção pelo próprio mesotélio induzir o remodelamento nas estruturas vizinhas. Contudo, são múltiplos os eixos de sinalização presentes no remodelamento peritoneal, sendo que actualmente se investiga a importância de polimorfismos em determinados

eixos como o RAS e variações de receptores, de maneira a explicar as diferentes alterações observadas nos doentes submetidos a DP e os diferentes perfis de transporte.

Deste modo, à luz do conhecimento actual que as aquaporinas estão envolvidas não só no processo ultrafiltrativo mas também em fenómenos como angiogenese e migração celular, as aquaporinas podem ter uma participação no processo fisiopatológico do peritoneu para além da sua função na ultrafiltração de água. Presumivelmente, os mesmos mecanismos lesionais envolvidos no remodelamento podem afectar as aquaporinas, tendo se proposto possíveis mecanismos e alvos.

Do mesmo modo, a influência das próprias aquaporinas na biologia da célula não é completamente clara, uma vez que os mecanismos de regulação não são completamente conhecidos.

Deste modo as aquaporinas apresentam-se como um atraente alvo terapêutico, contudo até a data não foi descoberto nenhum antagonista com capacidade de ser utilizado *in vivo*. Por sua vez, os agonistas constituem uma hipótese teórica, porém nenhum composto com acção directa sobre o canal. De salientar a transcrição aumentada obtida por Arteaga et al, que através de glucocorticóides obteve um maior numero de AQPs na membrana e subsequente aumento da taxa de UF em doentes seleccionados.

Os mais recentes avanços em termos de técnicas de screening de fármacos e simulação computacional irão acelerar o processo de descoberta de potenciais fármacos.

Novas linhas de investigação são necessárias para clarificar a real importância das aquaporinas.

As aquaporinas representam assim o ultrapequeno poro previsto por Rippe mas também uma nova janela sobre o processo fisiopatologico da barreira peritoneal e da própria fisiologia humana.

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ABBREVIATIONS AND ACRONYMS

ACEi – angiotensin conversion enzyme inhibitor

AGE – advanced glycosylation endproducts

APD- automated peritoneal dialysis

AQP- aquaporin

ARB – angiotensin receptor blocker

CAPD – continuous ambulatory peritoneal dialysis

CHIP28 – channel forming integral protein 28

ESRD - end-stage renal disease

FWT - free water transport

GDPs – glucose degradation products

GFR- glomerular filtration rate

MC- mesothelial cells

NOS- Nitrous oxide synthase

NPA- sequence of 3 aminoacides (Asn-Pro-Ala) highly conserved in aquaporins

PD - peritoneal dialysis

ROS- reactive oxygen species

RAS- renin angiotensin system

SD – standard deviation

TEA - tetraetylammonium

UF - ultrafiltration

UFF- ultrafiltration failure

VEGF- vascular endothelial growth factor

I – AQUAPORINS

- Discovery and Definition
- Structure of Aquaporins
- Water Permeation and Small Solute Transport
- Regulation of Aquaporins

Since their formulation as theoretical hypothesis in the 80´s[1] until the demonstration in 1992 of CHIP28 as an water channel by Preston *et al*[2]*.,* the aquaporins are a scientific subject of intense research and the simplistic view of a passive water channel has evolved and today they are linked to innumerous physiological processes from embryogenesis to cancer biology.

Aquaporins are an ancestral family of transmembranar proteins (channels) divided until now in three groups[3], by their ability to facilitate the passage of water, small solutes and glycerol in: orthodox aquaporins, aquaglyceroporins and unorthodox aquaporins. Their presence and distribution seems to be universal, being present in all living beings, animals and plants.[4] When isolated from the rest of the other aquaporin subfamilies, the mammalian AQP's are a group composed of 13 different members with functional and phylogenetic differences conditioned by their genetic coding and ultra-structure.

Structurally they are a tetramers composed of four monomers in which six helical segments and respective loops of variable length connect with each other to form the basic subunit, the monomer[5] (Fig.1-A). The connection between helices gives rise to the AQP fold and the NPA motif that is localized in the center of the fold, the stability of this fold is provided by the packing of helices and by tetramerization (Fig.1-B).

The orientation of the helices and the exposed extracellular domain condition the conformation[6] of the channel and his selectivity to water. The channel can therefore by divided in extracellular and cytoplasmatic vestibules and pore. Each monomer functions as an independent pore, so that one aquaporin is actually constituted by four pores.

The channel characteristics, with a diameter of the vestibule of 2.8A and two constriction zones[7] (ar/R and NPA motif), make the channel selective to water by size and by creation of an energetic barrier by the dipole moments of helices B and E which impedes the penetration of protons. The energetic cost of

transporting water along the membrane is compensated by the interactions of water with “walls” of the pore, lowering the energy of the system[8].

The geometry and structure of the pore are major factors in the function of aquaporins[9], since changes in critical residues at pore entrance and channel itself alter drastically the pore conductance and selectivity, resulting even in total obstruction of the pore and as result a non-functioning channel. This fact has been used as basis to experimental protocols[10] that induce mutations in specific sites to test the relevance of a given residue, being particularly useful in explaining differences in permeation between different AQP’s. Changes in the structure induced by external factors are termed *gating,* and it gives rise to a different conformational status and permeability of an AQP[11].

Based on these differences between isoforms of aquaporins they are, as mentioned before, grouped in aquaporins that exclusively permeate water and aquaglyceroporins, permeable to water, glycerol and urea (AQP 3, 7, 9 and 10) based on the striking difference between pore diameters 2.8A vs 3.4A, respectively. AQP9 is capable of transporting amino acids, small polar solutes and even sugars. Other small molecules and gases such as CO_2, NH_3, NO and H_20_2 are small enough to pass the pore, but due to the intrinsic high permeability of the phospholipid membrane to gases the biological impact is thought to be minimal. Presently is in under intense discussion the possibility of AQP1 be a multifunctional channel *in vivo*, this hypothesis is gaining further evidence and is based on the ability of the central pore to conduct cations under special conditions[12-14]. It physiological meaning is still obscure with proposed roles in signal transduction, mechanical compliance to pressure, organelle volume regulation and cell volume in migration.

The regulation of AQP’s is still a debated question, where the gating, trafficking and transcription of AQP’s pose as the main mechanisms for controlling cell water homeostasis[15]. Since AQP1 is constitutionally expressed in the membrane and the abundance of it (via transcription and expression) controls the tissue/cell response to the environment, this axis of control doesn’t provide the explanation for rapid adaption to changes of the extracellular environment. The gating mechanism could partially explain this by coupling of water transport to cell signaling and metabolism. Gating is proven in plant

AQP[16,17], but not widely accepted in human AQP's and its potential meaning in such a dynamic system is difficult to predict[15].

AQP2 trafficking induced by vasopressin in the kidney presents as one of most studied models[18,19] and could provide clues to the key mechanisms in AQP1 control. Here the AQP2 and AQP3, present in the collecting duct are responsible for the permeability of this segment by responding to a rise in vasopressin concentrations which binds to the V2 receptors in the basolateral membrane which cause a cascade of reactions with activation of protein kinase A (PKA) and the migration and fuse of AQP2 vesicles to the membrane, augmenting permeability to water. Long term regulation is achieved when circulating vasopressin are increased for a long period of hours resulting in increased transcription. Recently, *Conner et al*[20,21] demonstrated that rapid translocation of AQP1 in response to a hypotonic stimulus induced a intracellular calcium elevation, activation of calmodulin and phosphorylation of AQP1 with effective translocation within 30s. This could represent the missing link between rapid volume changes and AQP status.

II- ROLE OF AQUAPORINS IN HUMAN PHYSIOLOGY

- Presence and Distribution in the Human Body
- Function and Relevance in Different Organs
- Non Predicted Functions of Aquaporins
- Emergence of a new disfunction: Aquaporinopathies
- Diagnosis of AQP disfunction

The presence and distribution of AQP's varies according to the organ, but there is an universal distribution in human epithelia and endothelia, fulfilling the anticipated role in water transport and gland fluid secretion; other roles like cell migration and neural signaling were proposed after observation of impairment in *KO*-mice.

In epithelial cells the exchange of water across barriers was expected, and can be performed in two different ways[22]: active near-isosmolar fluid transport, as in the kidney proximal tube absorption and acinar epithelium secretion of salivary glands; and an active fluid transport across epithelia in which the AQP amplifies the fluid transport caused by an osmotic gradient actively generated by solute transport. AQP1 has a critical role in transepithelial water transport in presence of a significant osmotic gradient as demonstrated by the AQP1 null mices which present severe polyuria and impaired urinary concentration. In fact AQP1 function in the thin ascending limb of Henle and collecting duct maintains the countercurrent multiplication mechanism and exchange resulting in a hypotonic medulla and diminished reabsorption by the proximal tube. In rare, AQP2 mutation in humans the resulting disease is a nephrogenic diabetes insipidus, with leading symptoms of polyuria and lack of urine concentration ability.

In the brain, AQP4 presents itself as bidirectional water channel facilitating fluid transport according to the nature of the stimuli as demonstrated by Verkman *et al* in AQP4 null mices with clearly different outcomes: improved survival of null mices in cytotoxic edema[23], with a flow driven by osmotic forces through an intact blood-brain barrier (impedance of flow into the brain) and poorer survival in vasogenic edema[24], where a disrupted blood-brain barrier leaks and drags water with it, which has little escape route since AQP4 is disabled resulting in greater brain edema. AQP4 is also expressed in supportive cells in electrically excitable tissues where it could influence neurotransmission,

since AQP4 null mice present with a variety of disturbances such as: impaired hearing, vision, olfact and reduced seizure threshold. This fact is elegantly explained by the hypothesis of a K^+ buffer role of supporting cells[25]. In this hypothesis, the supporting cells would regulate the extracellular compartment by controlling the amount of water and therefore the size of the extracellular space and the concentration of ions, like K^+. In the absence of AQP4 the compartment would contract and slow the K^+ re-uptake by the neighboring cells, therefore conditioning the excitability and conduction.

The experimental studies in mice also revealed unexpected participation of AQP's in some physiologic phenomena: cell migration, cell proliferation, fat metabolism and skin hydration[26].

Regarding cell migration, AQP1 deletion in mice resulted in impaired growth and vascularity in implanted tumors. On the other hand, modification of cell lines that don't normally express AQP resulted in increased migration when compared to the wild type.

"Aquaporinopathies" are defined as human diseases caused by aquaporin dysfunction, the two mechanisms known until now are loss-of-function mutations and auto-immune response against extracellular epitopes on AQP4.

Loss of function mutation are rare, but AQP2 mutation not-X-linked causes, as mentioned before, NDI, with an estimated incidence of 1in 20million births. The causative defect is an abnormal protein folding with retention in cytoplasm and plasma membrane targeting. The AQP0 thought of having an adhesion function in the lens fibers are the cause of some congenital cataracts due to AQP0 mutation. The association between AQP polymorphisms and disease and disease causing mutation of AQP's has not been described[27].

The neuromyelitis optica (NMO) who shares some common traits with multiple sclerosis is a disease with ocular and spinal commitment characterized by blindness and paralysis. Hallmark of this pathology is the presence of IgG directed against AQP4. In fact these auto-antibodies when administered in rats with previous neuroinflammation, cause NMO symptoms. Currently, based on experience from oncology, with monoclonal antibody target therapeutics like trastuzumab, an NMO antibody is being developed to block the IgG-AQP4[28].

AQP based diagnostics is still a undeveloped field, with much of the functional weight of polymorphisms not explored in depth and with the role of AQP's in

other disease conditions not clear. The role of AQP's in a specific disease needs to be clarified in order to be valuable in the clinical set. Exceptions are made for AQP based assay of serum antibodies in NMO, immunoreactive protein in urine to NDI and possible interest in AQP specific antibodies in skin and salivary glands immune diseases; AQP1 detection exams for proximal tubule injury. Parallel to this, the possibility of characterizing pathology specimens for AQP presence seems to be particularly promising in tumors, based on recent correlations of tumor grade and AQP expression[29,30].

III- AQUAPORINS AND PERITONEAL DIALYSIS

- The peritoneal membrane as a transport barrier
- Impact of aquaporins in peritoneal exchange
- Aquaporins and Ultrafiltration failure
- Peritoneal Pathophysiology
- Putative role of AQP's in peritoneal fibrosis and inflammation

Peritoneal dialysis together with hemodialysis represents the available renal replacement therapies for patients with end-stage renal disease (ESRD), defined by K/DOQI as a renal function with GFR inferior to 15mL/min/1,73m^2. ESRD is a growing health problem with an estimated incidence of 2,786 million patients worldwide and with a 6.7% annual growth rate, in 2011.

For its relative simplicity, lesser impact in the daylife of patients and lower costs in comparison to hemodialysis, PD is an attractive therapy[31]. The outcomes of both are considered equal, but PD presents an early survival advantage during the first years of therapy and to an extent it can be even greater depending on the burden of comorbidities.

Peritoneal dialysis consists in using the peritoneal cavity and respective membrane as an interface of transport between circulating blood and a dialysate solution, in order to remove metabolites, toxins and water, therefore replacing kidney function. In practice this technique implies instillation of dialysate through a catheter, a resting period of hours and a final drainage. The manipulation of the system can be performed by the patient himself, referred as continuous ambulatory PD (CAPD) or by a mechanized device referred as automated PD (APD).

The transport of solutes and water is obtained by the creation of a concentration and osmotic gradient through the membrane, with the use of dialysate with an osmotic agent (commonly glucose) and physiological concentrations of sodium, chloride, calcium, magnesium and a buffer to stabilize the pH. This way water is extracted by osmosis, small solutes by diffusion and macromolecules by convection (explored further ahead).

The peritoneal cavity used in the procedure is of paramount importance but the concept of anatomical peritoneum and peritoneum as a transport barrier differs: the anatomical peritoneum is defined as the serosal lining of the abdominal cavity, the mesothelium[32]. The concept of transport barrier is far

more complex, and includes the mesothelium, the interstitial matrix beneath it and the capillaries (Fig.2-A).

Therefore the total anatomical surface area does not correspond to the functional surface area[33] that compromises the peritoneum involved in the transport. The last depends on the arrangement of the capillaries in the interstitium: density, surface area and distribution, so that to a given surface area of peritoneum, only a portion will be in contact with the dialysate and of this portion only a fraction will meet the requirements to an effective transport.

The mesothelium composed of a single layer of mesenchymal cells, with their own basement membrane and glycocalix, is responsible for lubrication of the serosa by secretion of phospholipids and glycosaminoglycans, preventing adhesions and has major *pivotal* role in host defenses. Nevertheless, it doesn't represent a major barrier in transport, since no significant alteration was observed in the transfer rates in mice submitted to total peritonectomy and in patients with peritoneal carcinomatosis[34].

On the other hand, the interstitium is a matrix of amorphous substance of high molecular weight interlaced with bundles of fibres and cells (adipocytes, fibroblasts and occasionally monocytes), that contains also the arterial, venous and lymphatic vessels (mainly capillaries) and nerves. Since it constitutes the pathway between blood-dialysate, it is considered to be one of the two barriers of transport. The thickness and negative charge of the interstitium, are considered to account for the diffusion of both small solutes and macromolecules, since the thickness represents the length the solute most travel and a selection of macromolecules is made based on their charge (repulsion).

The microvasculature is composed of true capillaries (Ø 5-6 μm) and postcapillary venules (Ø 7-20 μm). The capillaries are classified as continuous, with endothelial cells anchored to a basement membrane and closed together by adhesion junctional proteins. This layer is then encircled by a glycocalix. The endothelia besides is role in transport, actively secrets auto regulatory substances that control the tonicity of the vessels, like NO, EDHF and ET peptide family in addition to other promoters and growth inhibitors and other compounds involved in thrombogenesis, fibrinolysis and leukocyte adhesion.

The capillary wall of these vessels is considered the main barrier to exchange process and functionally has the behavior of an heteroporous structure composed of three pore sizes: large (200-400A), small (40-65 A), and ultrasmall (4-6A), demonstrated by Rippe *et al*[35] as an accurate predictive model of transport. The pore size is intimately related to their selectivity and to forces that drive solutes through them (Fig.2-B): the large pore is permeable to macromolecules, small solutes and water where the predominant force is hydrostatic pressure; the small pores are permeable to small solutes and water and impermeable to solutes with molecular weight above 69.10^3 Da with hydrostatic and osmotic pressures as predominant forces[33]. The ultrasmall pore is a transcellular pore with osmotic pressure of low molecular weight solutes as drive force and permeability to water molecules only, later proven to be aquaporins (to be developed further ahead). The morphological equivalents to small pores are interendothelial clefts and large pores are believed to be larger than average interendothelial clefts (looser interendothelial adhesions).

The transport in the barrier can therefore be divided in fluid transport and solute transport. Regarding fluid transport, it is considered tri-phasic: an initial net ultrafiltration with effective osmotic pressure on AQPs and passage of water; an isovolemic phase, with counterbalance of ultrafiltration through absorption and a final phase of net fluid absorption.

Some factors influence the total amount of ultrafiltrated water, to be mentioned: the osmotic gradient start to decay as a result of the absorption of the osmotic agent, usually a small solute; a part of the water filtered into the peritoneal cavity is absorbed by influence of an elevated hydrostatic pressure into the lymphatic drainage, mainly stomata, a subset of lymphatic structures and into the adjacent tissues and also by backfiltration through the small pores.

Paradoxically, under normal circumstances the blood flow rate is not a decisive variable as in other organs, but rather, as previously mentioned, the perfusion rate, the surface area of capillary available for transport (increased with vasodilation) and the recruitment of other microvessels. Recently, another factor was proposed by Stachowska-Pietka *et al*[36], with results comparable to the actual model, in which spatial distribution of the capillaries also interferes with the exchanged based on the interaction of hydrostatic pressure and effective range of the osmotic pressure. As a result, not all capillaries are

involved in transport but only the closest to the peritoneum can be subject of the created gradients and participate in transport, resulting in a thin layer of effective vascular recruitment.

Secondly, the solute transport is effectuated by solute diffusion according to the differences in concentrations between blood and dialysate (Fig.2), and by convection, the solute dragged with the water flux. Diffusion rate is proportional to the concentration gradient, the solute diffusion constant, and the effective surface area and inversely proportional to the diffusion distance. In clinical practice, knowing the volume flow and sieving coefficients, diffusion is easily calculated through the mass transport coefficients across the barrier (MTAC), based on initial and final concentration of solute in plasma and dialysate.

Ultrafiltration and its product, free water, are very important in PD. Ultrafiltration is a predictive marker of survival, with a cumulative risk for permanent loss of UF after 1 year of 3% and after 6 years, of 31%, has reported by Heimbürger *et al.*[37] As mentioned, the aquaporins are the morphological translation of the ultrasmall pores, and they are responsible for a high percentage of FWT in PD[38].

Cumulative data, from AQP1 null mice, began to demonstrate AQP1 as the ultrasmall pore, where Yang *et al*.[39] registered a decrease in cumulative UF when exposed to a hypertonic solution. Further evidence was provided by Ni *et al*[40], that demonstrated severe water transport dysfunction albeit appropriate osmotic charge. The 50% loss of UF was in line with the predicted AQP transport by the three pore model. Although not so dramatic, the mice with intermediate phenotype also presented impaired water transport. Previously, Carlsson *et al.*[41] demonstrated the presence of AQP1 expression in the peritoneum and specific inhibition of 66% with $HgCl_2$.

These findings, corroborated some clinical observations of dissolution of sodium in dialysate during the first hour of dwell, creating a graphic dip in the sodium concentration latter attributed to the water transport through ultrasmall pores. This sodium dipping is now considered an indirect measure of UF.

The inverse situation of increased expression and subsequent rise in UF was also demonstrated, this time Stoenoiu *et al.*[42] based on the presence of glucocorticoid elements in the AQP promotor gene induced an over expression

of AQP1 in mice with high doses of glucocorticoids with increased UF as result. Latter Arteaga *et al.*[43] tried a similar approach in selected patients with promising results: an almost 2 fold increase in sodium dip and ultrasmall pore-specific UF.

In clinical practice, AQP function and contribution to UFF can be quantified by novel protocols of peritoneal transport. In practice, the patient is submitted to an individual assessment of his peritoneum transport characteristics and his transport profile is included in one of four distinct transport groups[32] according to the obtained D/P creatinine: Fast transporter (above 1 SD); Faster than average transporter (between the mean and the superior SD); Slower than average (between the mean and the inferior SD) and Slow transporter (below -1SD). The commun peritoneal equilibration test (PET) was standardized by Twardowski *et al*.[44], and consists in a four hour dwell with a dialysate solution of 2.27%/2.5% glucose, after a previous long dwell (8-12h). Samples of the effluent and dialysate bag are taken in the beginning and the 10, 30, 60, 120 and 180 min. Serum samples are collected at the end. Measurements of solutes in samples are made, including: sodium, potassium, urea, glucose, creatinine and total proteins. The D/P ratios are calculated with the measured values.

Other variations of PET were elaborated, worth mentioning: the fast PET, a simplified version of PET with only one sample per body fluid and measurement of only urea and creatinine; and the Mini-PET[45], proposed by La Millia *et al,* that consists in 1 hour dwell with a 3.86/4.25% glucose solution, assuming maximal free water transport with this osmotic gradient. Therefore it allows the calculation of FWT, a feature not present in standard PET and the possibility of distinguish net ultrafiltration changes due to small solutes or to FWT. In 2012, Bernardo *et al*[46] proposed a different protocol based in the standard PET and mini-PET, in which an interim step was added to the sPET (total drainage at 60' and weight of the ultrafiltrated water) which allows a more accurate and direct estimation of the NUF.

Although AQP dysfunction can be a cause of UFF, UFF is a complex clinical situation with multifactorial causes and results in a functional fluid overload. UFF is defined by International Society of Peritoneal Dialysis as NUF inferior to 400 mL after a 4h dwell with a glucose solution of a 3.86/4.25%.

This can be attributed to high fluid intake, non-compliance, un-optimized prescription and low drained volume. The latest can be due to technical fault in the drainage system or peritoneal membrane failure. Peritoneal membrane failure includes several causative mechanisms to a low drained volume and according to patient transport status different causes can be suspected: in a slow transporter, disruption of the peritoneal space; in a fast transporter, inherently high transport, recent peritonitis and long term PD; finally in the intermediate transporter, technical fault, enhanced reabsorption and AQP deficiency. The pathophysiology will be addressed in more detail in the following section.

Loss of peritoneal function is a dramatic outcome for patients in PD, since the treatment failure is up to 50% in patients with more than 6 years of therapy. Although this risk can be minimized with updated solutions and protocols.

The use of peritoneum as dialytic membrane is an unnatural role for the living structure, and since the beginning of PD architectural alterations of the membrane can be found. The major causes of drop-out in long term PD are infections and the membrane failure, which in extreme cases can lead to a generalized peritoneal sclerosis and the establishment of encapsulating peritoneal sclerosis, herald of a poor prognosis[47].

This way peritoneal remodeling is a deleterious adaptation of the peritoneal membrane with structural and functional alterations in response to a variety of insults, acute (e.g. peritonitis) or chronic (e.g. PD itself). These induced alterations translate into modifications in the cell microenvironment and populations, with signaling pathways shifted towards inflammation, angiogenesis, remodeling (sclerosis) and recruiting of effector cells.

As mentioned, since the first PD alterations in the membrane can be found, the mesothelium presents depopulated areas as a result of mesothelial cell loss, with punctual zones of high mesothelial density (i.e. regeneration process). Among these, vimentin positive cells, indicative of on-going endothelial to mesenchymal transformation, are present. The submesothelial layer shows progressively increased thickness, and infiltration by inflammatory cells. The increased thickness attenuates the effective osmotic pressure on capillaries. The angiogenesis and lymphangiogenesis results in an incremented number of

blood vessels that correlates with higher vascular area, faster absorption of glucose resulting in a decrease of osmotic pressure by early dissipation with a lower UF as final result. The lymphatic expansion produces enhanced reabsorption, further contributing to lower UF. Also, the loss of adhesion proteins induced by inflammation and remodeling results in larger vascular interendothelial gaps, with loss of proteins. A shift in the imunne cell population is seen with predominance of neutrophils and in more chronic cases activated macrophages, with a higher than normal presence of milky spots (i.e. accumulation of macrophages). Animal studies show that in early PD, these alterations are a local phenomenon spread in clusters around the cavity, but with time and continuous stimulation, the lesions coalesce and become generalized. It is commonly accepted that the peritoneal remodeling resembles a chronic low-grade inflammation process.

There are multiple causes that trigger peritoneal response and remodeling, such as the PD catheter, presence of PD solution in the cavity, the composition of the dialysate, uremia[48] and peritonitis[49]. Their contribution and ability to trigger a response is variable among each other, but due their common presence in PD patients they are thought to act synergistically by activating the same signaling pathways.

The PD catheter has been demonstrated to cause local response in the site of insertion; the PD solution causes physically induced stress in the mesothelium layer, with morphological alterations. Uremia in the other hand induces hyperemia and inflammation contributing to the overall vasculopathy.

The composition of the dialysate causes inflammation via its lactacte, pH, and glucose content. The far most important aggressor is glucose and the products derived from it such as glucose degradation products (GDPs) and advanced glycation end-products (AGES) created during heat sterilization of solutions. GDPs and AGEs induce VEGF and TGF-β synthesis by MCs and they are considered the main mediators of peritoneal remodeling. Besides inducing oxidative cell stress[50], the activation of AGE receptors (RAGE)[51] is linked to activation of multiple signal-transduction pathways[52], some of which involved in angiogenesis and fibrosis. The blockage or absence of these receptors results in significant reduction of EMT and fibrosis in mice models.

Peritonitis constitutes an acute event of massive inflammatory activity with release of multiple pro-inflammatory cytokines, with acute loss of UF, marked vascular proliferation and inflammatory infiltrates. Although the alterations are considered reversible[53], sustained bacterial peritonitis can lead to irreversible loss of UF and permanent damage of the barrier. As demonstrated by Devuyst *et al*.[54] in mice, the NOS plays an important part in the vascular changes during the event. Of the three isoforms present in the membrane – iNOS, eNOS and nNOS – the eNOS isoform controlled by intracellular Ca^{2+} levels, is responsible for the alterations in profile transport due to its vasoactive nature, with marked vasodilation, which allows greater absorption of glucose and dissipation of osmotic gradient, an enhanced infiltration of neutrophils and loss of proteins. The deletion of this isoform is accompanied by a reduction of these alterations.

Despite the fact that the mesothelium is not a barrier for transport, it represents a keystone in peritoneal response to PD and infection, being responsible for the production cytokines and inflammatory mediators, in response to the mentioned external factors and to stimuli from other peritoneal cells. It is also capable of undergoing transdeferentiation, in an attempt to restore the loss of other mesothelial cells, in a process named endothelial-to-mesenchymal transition (EMT)[55,56]. In this process, the cell starts to suffer a detachment of the underlying basement membrane, and it's transformed into a fibroblast like cell with enhanced migratory and fibrogenic abilities[55]. This process is sustained by the presence of TGF-β[57,58]. In fact this growth factor is not only responsible for EMT, but also for the capability of mesothelial cells to activate myofibroblasts (i.e. fibrosis), vasculopathy[59] and deposition of submesothelial layer[60] since TGF-β induces MCs release of VEGF, FGF and other growth factors.

The mesothelium repairing process is composed of three proposed mechanisms with still undefined weight of contribution, but thought to act in a complementary way: implementation of free mesothelial cells in suspension[61]; MCs at the border of denudated zones migrate to fill the gaps; and new MCs originate from the submesothelial layer and migrate to the damaged area. It worth mentioning that for this EMT ability, the mesothelium is considered a source of stem cells[62] and it's autologous transplantation is considered by some authors as an therapeutically option in PD[63].

The RAAS system is recently being subject of investigation, based on the known effects of angiotensin in regulating cellular proliferation, apoptosis and fibrosis, with general profibrotic profile. Some tissues have the capability of producing all components of the RAAS axis, and this seems to apply to the peritoneum since MCs appear to have RAAS codifying genes has demonstrated by Nessim *et al*[64]. Glucose and the augmented hydraulic pressure induce activation of RAAS in MCs and subsequent production of angiotensin, with regulatory function in cell proliferation, apoptosis and fibrosis. These effects are achieved by the induction TGF-β / fibronectin production and ultimately of VEGF. This affects not also the vasculature with increased permeability, vasodilation and angiogenesis but also fibrosis. Recent attempts of using ACEi and ARB as a preventive measure against loss of UF, seem to have positive results: in rats, Duman *et al*.[65] demonstrated preserved UF and significant decrease in VEGF levels when treated with a intraperitoneal dose of enalapril. Additionally, a retrospective study demonstrated maintenance in solute transport status in patients treated with oral ACEi/ARB. Pérez-Martinez *et al*.[66] recently demonstrated in mice, the benefits of aliskiren in UF preservation and protection of MCs against glucose oxidative stress with lesser proapoptotic molecules when compared to control.

The complexity of peritoneal remodeling is also related to numerous activated signaling pathways. The conjunction of these result in the observed alterations in the peritoneum and an accelerated mesothelial cell cycle. So far the main pathways identified are COX2, p38MAPK, ROS, RAGE, JAK-STAT and Tyrosine Kinase Receptor pathway[67,68].

In fact, based on the current knowledge of peritoneal remodeling and the different transport profiles, it is suspected that individuals subject to PD have different levels of remodeling and baseline transport, not only due to pre-PD comorbidities but for the presence of polymorphisms in RAS, RAGE, VEGF, TGF-β which could result in an amplified response to PD. These are currently under investigation.

Based in the current knowledge of AQPs, their importance is established in vascular endothelia and UF, as the hypothesized ultrasmall pore. But the recent advances in the field of aquaporins render more questions since they appear to be involved in innumerous physiological processes. Considering that the

mesothelium is subject to intense oxidative stress with ROS formation[50] and exposure to GDPs, are the intracellular domains of aquaporins damaged and subsequently the pore conformation affected? It's known that relation between pore geometry and water conductivity is critical, a single alteration can lead to drastic changes in water transport. Similarly other points of the aquaporin life cycle can be target of damage such as the *folding* as previously mentioned.

Regarding the control of aquaporins in the cell, as highlighted before not all control mechanism are yet clarified, but based on the rapid translocation which uses intracellular calcium and PKC as effectors, can other pathways be involved in increased translocation? As calcium is a common second messenger[69,70] to other pathways this may represent an opportunity for up-regulation of translocation via "indirect" pathway.

Also noteworthy is the fact that many signaling pathways are present in peritoneal remodeling but their final effect on the AQP status is not clear. Many signaling pathways translate into different intracellular mechanisms. Is there a prevalent pathway or the end-result is the conjunction of all stimuli? The answer might be related to MicroRNA. MicroRNA regulates mRNA expression by altering transcription and translation. They are small non-coding RNA molecules, well conserved in eukaryotic cells and one single miRNA can have multiple targets (mRNA). Altered expression was observed in some diseases. In other models of disease, microRNA 320a was presented as an endogenous modulator of AQP1 and AQP4[71-73] with possible interference in other cell processes, but the fact remains elusive.

Also, given the ability of AQPs to alter the intracellular concentrations (by dilution), can they influence the cell function and intracellular pathways?

AQP1 is present in vascular endothelia and in mesothelium. In the vascular endothelia, AQP up-regulation is considered beneficial since it contributes to a higher UF. On the other hand, Verkamn *et al.*[74,75] reported impaired cell migration on AQP null mice. So, since AQP1 may also play a role in migration of inflammatory and mesothelial cells, up-regulation can translate into an enhanced pathological process.

AQP might also influence apoptosis. As highlighted by some authors like Santamaria, Selgas, and Gotliob the mesothelium is subject of an accelerated life cycle, in which apoptosis controls the number of cells and their viability by

removal of undesirable cells and its control represents a therapeutic opportunity[76-78]. Apoptosis could be defined as a controlled cellular implosion with successive destruction of intracellular organelles, as a response to internal and external factors in which is included environmental stress[79]. Some authors postulate an involvement of AQPs in apoptosis, based in the diminished rate observed in AQP null cells. Recently Lee *et al.*[80] hypothesized a role for AQP in mitochondrial fission after observation of impaired fission in presence of Hg^{2+}.

On the other hand Jablonsky *et al.*[81] demonstrated Hg^{2+} based inhibition of AQP1 in blocking the apoptotic processes such as apoptotic cell volume decrease (AVD), DNA degradation and caspase3 activation in thymocytes and granulosa cells from the ovary. The overexpression of AQP1 resulted in enhanced apoptotic process. This volume depletion is proposed to facilitate the reduction of intracellular K^{+}[82,83] and the initiation of the apoptotic program.

It seems that the apoptotic response involves a coordinate control of intracellular and membranar aquaporins, that together compose an axis of cellular volume control mediated by an unknown mechanism.

- Antagonists
- Agonists
- Trends in Research

Excluding the monoclonal antibody Anti-IgG AQP4, so far the compounds can be divided in antagonist and agonists.

The pharmacological antagonists of AQP are transition metals (mercury, silver[84], gold[84]), quaternary ammonium compounds[85] (tetraethylammonium (TEA), sulfonamides and related compounds (acetazolamide)[86] and phloretin[87]. The direct effect of these compounds is validated through residue site-directed mutagenesis: the suspected target of the inhibitor is altered via mutagenesis and substituted by another residue. In theory, the inhibitor loses his binding site and his ability to diminish water flow. If that's the case the mutated residue is identified as a connection site of that compound.

Transition metals, mercury in particular where the first known inhibitors followed by silver and gold but the role of these compounds is restricted to laboratory environment due to their high toxicity *in vivo*. Bearing in mind the similarities between AQPs and ion channels, it was at the time, suggested the ion channel blockers could also affect AQP. TEA, a pore-occluding K^+ blocker, has antagonistic properties due to its interaction with the tyrosine 186 residue at outer vestibule, but in line with the transition metals, it effect in vivo has undesirable consequences since it blocks a variety of K^+ channels, as such it is only used in an experimental basis. Phloretin is also mentionable for its dual inhibition profile, but unsuited for human blockage.

Sulfonamides, a group that includes antiepilectic drugs is target of research, but with unconfirmed results *in vivo*. Also based on the low inhibition caused by bumetanide, a screening of chemical structure library was performed with bumetanide chemical structure as basis. This resulted in the discovery of AqB013[88] that simultaneously blocks AQP1 and AQP4.

The screening of the National Cancer Institute Small Molecule Collection with the novel fluorescence method result in 4 positive results: NSC164914, NSC670229, NSC168597 and NSC301460.Particularly interesting is the second molecule for its simple structure and reasonable inhibitory effect[89]. Recently,

three undisclosed compounds were considered inhibitors with no chemical similarity with the known compounds[90].

The existence of agonists is closely connected to *gating,* presuming a non-maximal AQP conformation and possible optimization[91]. The theorized mechanism is the stablilization of the open conformation state of the pore by a ligand. The available ligand-sites proposed by docking simulations have been identified in the intra and extracellular domains[92].

So far the only therapeutic measure that in fact increased AQP action was based on an indirect mechanism, since AQP1 gene contains glucocorticoid response elements, Arteaga *et al.* demonstrated an increased in UF due to quantitative rise of aquaporins[43].

The modulation of aquaporins is an evolving subject but despite all the efforts currently undertaken some obstacles such as the lack of natural endogenous ligands and the poor adequacy of heavy metal based assays to detect small organic compounds need to be surpassed in order to accelerate the drug discovery process. Great amount of information about possible binding sites and effectiveness is being given by computer modulation of AQPs and it represents a valuable source of information.

The advances in the automated cell assay[93] provided the capability of even faster and higher output and the screening of library compounds with effective results. Although better, it still doesn't reflect the complexity of intracellular mechanisms and the possibility of indirect modulation; new models and greater knowledge of the AQPs "life cycle" are needed in order to accommodate these possibilities. The recent advances in AQP function as a cationic channel[94] may also give rise to other therapeutic classes once its importance and role in pathophysiology is established.

V- CONCLUSIONS

Peritoneal dialysis represents a viable and attractive form of renal replacement. It is clear that the solution for a more effective and long-lasting technique are more biocompatible solutions and remodeling blockers. This is only possible with greater insight of the peritoneal pathophysiology.

AQP represents the ultrasmall pore and is responsible for the UF and FWT observed in PD. In clinical practice the quantification of AQP function, UF translates into a deepest and more knowledge about patient transport profile.

Other roles for aquaporins in the pathophysiology of the peritoneal barrier are currently under investigation, but without a doubt they are a part of the observed alterations and pathological processes. Their study will bring further knowledge relevant not only to PD, but to human physiology and cell biology.

VI- FIGURES

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